Docket No. 25401-25

**PATENT**

**CERTIFICATE OF MAILING**

I hereby certify that this paper is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Mail Stop Appeal Brief-Patents; Commissioner for Patents; P.O. Box 1450; Alexandria, VA 22313-1450 on October 27, 2004.



**IN THE UNITED STATES PATENT & TRADEMARK OFFICE**

| | | | | |
|---|---|---|---|---|
| Appellant: | Ib Mendel-Hartvig et al | : | Paper No.: | |
| Serial No.: | 09/582,734 | : | Group Art Unit: | 1641 |
| Filing Date: | October 6, 2000 | : | Examiner: | G. Counts |

For: **Analytical Method Comprising Addition in Two or More Positions and a Device and Test Kit Therefor**

## APPEAL BRIEF

Mail Stop Appeal Brief-Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

The present Appeal Brief is submitted in support of the Notice of Appeal filed by Certificate of Mail on May 24, 2004 and received by the U.S. Patent and Trademark Office on May 27, 2004.

## I. REAL PARTY IN INTEREST

The real party in interest in this appeal is the assignee of the present application, Pharmacia Diagnostics AB.

## II. RELATED APPEALS AND INTERFERENCES

There are no other appeals or interferences known to the Appellants, the Appellants' undersigned legal representative or the assignee which will directly effect or be directly

effected by or having a bearing on the Board's decision in the present appeal. However, the Board may consider copending appeals in Applications Serial Nos. 09/582,741 and 09/582,808 to be of interest.

## III. STATUS OF THE CLAIMS

Claims 1-4 and 6-35 are pending in this application and stand rejected. Claim 5 is cancelled. A copy of the claims on appeal is set forth in the Appendix.

## IV. STATUS OF AMENDMENT FILED SUBSEQUENT TO REJECTION ON APPEAL

An Amendment Under 37 C.F.R. §1.116 was filed by certificate of mailing on May 24, 2004, presenting a single change to claim 1 responsive to the rejection under 35 U.S.C. §112, second paragraph, newly made in the Official Action and presenting claims 2 and 19 in independent form. The Advisory Action dated July 13, 2004 indicated that the Amendment would not be entered, although no reasoning was provided for refusal of the entry of the Amendment.

## V. SUMMARY OF THE INVENTION

The present invention is directed to methods and devices for determination of an analyte in a sample by use of biospecific affinity reactants (specification, page 1, lines 1-7).

According to independent claim 1, the claimed method is for determination of an analyte in a sample in a flow matrix by use of a transport flow of one or more biospecific affinity reactants, at least one of which is analytically detectable (Reactant*) and one of which is firmly anchored in the matrix (Reactant I). The flow matrix comprises A) an application zone adapted for application of liquid (LZ), which liquid contains buffer and sample and optionally reactants needed for a complete determination, but not Reactant I, B) a

detection zone (DZ) with the firmly anchored reactant (Reactant I) located downstream of LZ, and C) optionally one or more zones in which any of the reactants needed for a complete determination, but not Reactant I, has been pre-deposited.

The flow towards the detection zone is initiated by addition of the liquid with sample in the application zone LZ for transport of analyte and reactants towards the detection zone (DZ), and the amount of the Reactant* bound to DZ is detected, wherein the detected amount is correlated to the amount of analyte in the sample. The flow matrix comprises at least two application zones for liquid LZ arranged substantially adjacent to each other:

$$LZ_m \ldots LZ_n \ldots LZ_1 \qquad DZ$$

$$\longrightarrow$$

flow direction $\longrightarrow$ wherein a) $LZ_n$ is an application zone for liquid, and n is the position of the application zone $LZ_n$, b) m is the total number of application zones in which flow is initiated and is greater than or equal to 2 and is not equal to n, with $LZ_m$ being the farthest upstream liquid application zone, c) one $LZ_n$ is an application zone for sample ($LZ_{n'}S$) and one $LZ_n$ is for Reactant* ($LZ_{n''}R^*$), with n" ≥ n'; d) $\longrightarrow$ is the direction of the flow, and e) DZ is the detection zone.

Flow is initiated by adding liquid to each zone $LZ_m$ . . $LZ_n$ . . $LZ_1$ in such a way that $\text{liquid}_{n+1}$, added to the application zone $LZ_{n+1}$, contacts the flow matrix substantially simultaneously and is transported through the matrix immediately after $\text{liquid}_n$ added to the nearest downstream application zone $LZ_n$.

The device of independent claim 18 is for determination of an analyte in a sample in a flow matrix by use of a transport flow of one or more biospecific affinity reactants, at least one of which is analytically detectable (Reactant*) and one of which is firmly anchored in the matrix (Reactant I). The device comprises a flow matrix having A) an application zone for liquid (LZ) containing buffer and sample and optionally reactants needed for a complete

determination, but not Reactant I, B) a detection zone (DZ) with the firmly anchored reactant (Reactant I) located downstream of LZ, and C) optionally one or more zones in which any of the reactants has been pre-deposited.

The flow matrix comprises at least two application zones for liquid arranged substantially adjacent to each other:

$$LZ_m \ldots LZ_n \ldots LZ_l \qquad DZ$$

$$\xrightarrow{\hspace{5cm}}$$

flow direction $\longrightarrow$ wherein a) $LZ_n$ is an application zone for liquid, and n is the position of the application zone $LZ_n$, b) m is the total number of application zones in which flow is initiated and is greater than or equal to 2 and is not equal to n, with $LZ_m$ being the farthest upstream liquid application zone, c) one $LZ_n$ is an application zone for sample ($LZ_{n'}S$) and one $LZ_n$ is for Reactant* ($LZ_{n''}R^*$), with n" $\geq$ n'; d) $\longrightarrow$ is the direction of the flow, and e) DZ is the detection zone.

The device is adapted, when flow is initiated by adding liquid to each zone $LZ_m$ . . $LZ_n$ . . $LZ_l$ in such a way that $liquid_{n+1}$ added to the application zone $LZ_{n+1}$, contacts the flow matrix substantially simultaneously to transport the $liquid_{n+1}$ through the matrix immediately after $liquid_n$, added to the nearest downstream application zone $LZ_n$.

Claims 2-4, 6-17 and 34 further define the method of claim 1, while claims 19-31 and 35 further define the device of claim 18. According to claims 2 and 19, n" > n', and claim 19 specifies that the device is intended for sequential transport of analyte and Reactant*. According to clams 3 and 20, n" = n' and claim 20 specifies the device is intended for simultaneous transport of analyte and Reactant*. According to claims 4 and 21, Reactant* is pre-deposited in its application zone ($LZ_{n''}R^*$). Claims 6 and 22 specify that $LZ_{n+1}$ is upstream and immediately adjacent $LZ_n$. According to claim 7, application of liquid is performed simultaneously in all $LZ_m$ . . $LZ_n$ . . $LZ_l$, all of the liquid application zones.

Claims 8 and 23 specify that $2 \leq m \leq 6$; n' is 1, 2 or 3; n'' > n; $LZ_{n'+1}$, $LZ_{n'+2}$, $LZ_{n'+3}$, $LZ_{n'-1}$, and $LZ_{n'-2}$ are application zones for liquids intended for transport of Reactant*, other reactant, or buffer without reactant. Claims 13 and 27 similarly recite that $2 \leq m \leq 6$ and n' for the application zone for sample ($LZ_{n'}S$) is 1, 2 or 3. Claims 9 and 25 recite that at least one of the zones $LZ_m$ . . $LZ_n$ . . $LZ_1$ comprises a pad or material layer applied on the flow matrix.

Claims 10 and 24 recite that the zones $LZ_m$ . . $LZ_n$ . . $LZ_1$ have zone spacers between each other. Claims 34 and 35 further recite that each zone spacer comprises a strip attached to the flow matrix.

According to claim 11, a composition of liquid flow from an application zone $LZ_n$ is not the same as a composition of liquid flow from the nearest adjacent application zone LZ in which flow is initiated.

Claims 12 and 26 recite that at least one reactant, other than Reactant*, is pre-deposited in an application zone $LZ_{n'''}R$ for liquid intended for transport of the reactant.

Claim 14 recites that Reactant* has biospecific affinity for the analyte so that Reactant* is incorporated into a complex Reactant'---Analyte---Reactant* in the detection zone in an amount related to the amount of analyte in the sample, in which complex Reactant' has biospecific affinity to the analyte and is (a) Reactant I, or (b) a reactant to which Reactant I exhibits biospecific affinity and which is transported from $LZ_{n'}S$ or from an application zone downstream of $LZ_{n'}S$.

Claims 15 and 29 recite that the flow matrix comprises at least one calibrator zone CZ. According to claim 15, the matrix calibrator is bound to, or in advance has been bound to, the matrix. According to claim 29, a calibrator or a binder for the calibrator is firmly anchored in the matrix. Claims 16 and 30 further recite that the calibrator zone or zones (CZ)

have a binder for the calibrator firmly anchored in the matrix, and calibrator optionally is pre-deposited in the matrix upstream of the calibrator zone or zones.

According to claims 17 and 31, the method and device, respectively, are for diagnosing allergy or autoimmune disease.

According to claim 28, the detection zone DZ comprises firmly anchored Reactant I, and a reactant to which Reactant I exhibits biospecific affinity optionally is pre-deposited in $LZ_{n'}S$ or in an application zone downstream of $LZ_{n'}S$.

Claim 32 recites a test kit comprising (i) a device according to claim 18, and (ii) Reactant*. Claim 33 specifies that the kit additionally comprises (iii) a calibrator when a binder for the calibrator is firmly anchored in the matrix.

## VI. ISSUES ON APPEAL

The following issues are on appeal for consideration by the Board:

A. The rejection of claims 1-4 and 6-17 under 35 U.S.C. §112, second paragraph, as being indefinite.

B. The rejection of claims 1, 3, 7, 9, 10, 13, 14, 18, 20, 21, 24, 25, 27, 28 and 32 under 35 U.S.C. §102(b) as being anticipated by the Dafforn et al U.S. Patent No. 4,981,786.

C. The rejection of claims 2, 4, 6, 8, 11, 19, 22 and 23 under 35 U.S.C. §103(a) as being unpatentable over Dafforn et al.

D. The rejection of claims 12, 15, 16, 26, 29 and 30 under 35 U.S.C. §103(a) as being unpatentable over Dafforn et al in view of the Robinson et al published PCT application WO 95/16914.

E. The rejection of claims 17 and 31 under 35 U.S.C. §103(a) as being unpatentable over Dafforn et al in view of the Self U.S. Patent No. 4,446,231.

F. The rejection of claims 34 and 35 under 35 U.S.C. §103 as being unpatentable over Dafforn et al in view of the Goerlach-Graw et al U.S. Patent No. 5,556,789.

## VII. GROUPING OF THE CLAIMS

With respect to the above-noted issues on appeal,

A. Appellants concede that claims 2-4 and 6-17 stand or fall together with claim 1 from which they directly or indirectly depend.

B. Appellants concede that claims 9, 14, 25, 27, 28 and 32 stand or fall with claim 1 or 18 from which they respectively depend. However, Appellants submit that claims 3, 7, 10, 20, 21 and 24 are independently patentable.

C. Appellants submit that claims 2, 4, 6, 8, 11, 19, 22 and 23 are independently patentable.

D. Appellants submit that claims 12 and 26, claims 15 and 29, and claims 16 and 30 are independently patentable.

E. Appellants concede that claims 17 and 31 stand or fall together.

F. Appellants concede that claims 34 and 35 stand or fall together.

Reasons in support of the independent patentability of the respective claims are set forth below.

## VIII. ARGUMENTS

As will be set forth in detail below, Appellants submit that the methods defined by claims 1-4 and 6-17 are definite to one of ordinary skill in the art. Appellants further submit that the methods, devices and test kits defined by claims 1-4 and 6-35 are neither anticipated by nor rendered obvious over Dafforn et al or the combination of Dafforn et al with any of the additional references cited by the Examiner. Accordingly, the rejections under 35 U.S.C.

§§ 102, 103 and 112, second paragraph, should be reversed. Favorable action by the Board is respectfully requested.

### A. The Method Claims are Definite

The rejection of method claims 1-4 and 6-17 under 35 U.S.C. §112, second paragraph, should be reversed as these claims are definite to one of ordinary skill in the art.

#### 1. The Examiner's Rejection

In claim 1, the Examiner asserted that it was unclear how the application zone was "adapted" for application of liquid.

#### 2. Claim 1 is Definite

Claim 1 recites that the claimed method is for determination of an analyte in a sample in a flow matrix by use of a transport flow of one or more biospecific affinity reactants, at least one of which is analytically detectable (Reactant*) and one of which is firmly anchored in the matrix (Reactant I). The flow matrix comprises, inter alia, A) an application zone adapted for application of liquid (LZ), which liquid contains buffer and sample and optionally reactants needed for a complete determination, but not Reactant I.

As the Examiner has not provided any detail as to why the terminology of claim 1 would be indefinite to one of ordinary skill in the art, particularly the transport flow matrix art. Accordingly, the rejection under 35 U.S.C. §112, second paragraph, is improper and should be reversed. Transport flow matrices are known in the art, as evidenced by the Dafforn et al reference cited by the Examiner. Various means may be provided for application of liquid to such flow matrices, for example in the form of ports and the like. Thus, one skilled in the art will recognize that the recitation in claim 1 that the application zone is adapted for application of liquid indicates that a port other means for applying liquid to the flow matrix is provided.

## B. The Claims are Not Anticipated by Dafforn et al

Claims 1, 3, 7, 9, 10, 13, 14, 18, 20, 21, 24, 25, 27, 28 and 32 are not anticipated by and are patentably distinguishable from Dafforn et al, whereby the rejection of these claims under 35 U.S.C. §102 should be reversed.

### 1. The Examiner's Rejection

The Examiner rejected claims 1, 3, 7, 9, 10, 13, 14, 18, 20, 21, 24, 25, 27, 28 and 32 under 35 U.S.C. §102(b) as being anticipated by the Dafforn et al. The Examiner asserted that Dafforn et al disclose an immunoassay device and method for determining an analyte in a sample wherein the device comprises a first means for introducing a sample into the device and a second means other than the first means for introducing a liquid reagent other than the sample into the device. The Examiner further asserted that Dafforn et al disclose that both of the application means are located upstream of an immunosorbing zone (detection zone) and that specific binding members are immobilized in the immunosorbing zone. The Examiner asserted that Dafforn et al disclose that the device contains dividers (spacers) between the first and second means and that the application of liquid can be performed simultaneously in the application zones, referring to column 24, lines 30-32.

### 2. The Claimed Methods and Devices Are Not Anticipated By Dafforn et al

According to independent claim 1, the claimed method is for determination of an analyte in a sample in a flow matrix by use of a transport flow of one or more biospecific affinity reactants, at least one of which is analytically detectable (Reactant*) and one of which is firmly anchored in the matrix (Reactant I). The flow matrix comprises A) an application zone adapted for application of liquid (LZ), which liquid contains buffer and sample and optionally reactants needed for a complete determination, but not Reactant I, B) a detection zone (DZ) with the firmly anchored reactant (Reactant I) located downstream of

LZ, and C) optionally one or more zones in which any of the reactants needed for a complete determination, but not Reactant I, has been pre-deposited.

The flow towards the detection zone is initiated by addition of the liquid with sample in the application zone LZ for transport of analyte and reactants towards the detection zone (DZ), and the amount of the Reactant* bound to DZ is detected, wherein the detected amount is correlated to the amount of analyte in the sample. The flow matrix comprises at least two application zones for liquid LZ arranged substantially adjacent to each other:

$$LZ_m \ldots LZ_n \ldots LZ_l \qquad DZ$$

$$\longrightarrow$$

flow direction $\longrightarrow$ wherein a) $LZ_n$ is an application zone for liquid, and n is the position of the application zone $LZ_n$, b) m is the total number of application zones in which flow is initiated and is greater than or equal to 2 and is not equal to n, with $LZ_m$ being the farthest upstream liquid application zone, c) one $LZ_n$ is an application zone for sample ($LZ_{n'}S$) and one $LZ_n$ is for Reactant* ($LZ_{n''}R^*$), with $n'' \geq n'$; d) $\longrightarrow$ is the direction of the flow, and e) DZ is the detection zone.

Flow is initiated by adding liquid to each zone $LZ_m \ldots LZ_n \ldots LZ_l$ in such a way that $\text{liquid}_{n+1}$, added to the application zone $LZ_{n+1}$, contacts the flow matrix substantially simultaneously and is transported through the matrix immediately after $\text{liquid}_n$ added to the nearest downstream application zone $LZ_n$.

The device of claim 18 is for determination of an analyte in a sample in a flow matrix by use of a transport flow of one or more biospecific affinity reactants, at least one of which is analytically detectable (Reactant*) and one of which is firmly anchored in the matrix (Reactant I). The device comprises a flow matrix having A) an application zone for liquid (LZ) containing buffer and sample and optionally reactants needed for a complete determination, but not Reactant I, B) a detection zone (DZ) with the firmly anchored reactant

(Reactant I) located downstream of LZ, and C) optionally one or more zones in which any of the reactants has been pre-deposited.

The flow matrix comprises at least two application zones for liquid arranged substantially adjacent to each other:

$$LZ_m \ldots LZ_n \ldots LZ_1 \qquad DZ$$

$$\xrightarrow{\hspace{6cm}}$$

flow direction $\longrightarrow$ wherein a) $LZ_n$ is an application zone for liquid, and n is the position of the application zone $LZ_n$, b) m is the total number of application zones in which flow is initiated and is greater than or equal to 2 and is not equal to n, with $LZ_m$ being the farthest upstream liquid application zone, c) one $LZ_n$ is an application zone for sample ($LZ_{n'}S$) and one $LZ_n$ is for Reactant* ($LZ_{n''}R^*$), with $n'' \geq n'$; d) $\longrightarrow$ is the direction of the flow, and e) DZ is the detection zone.

The device is adapted, when flow is initiated by adding liquid to each zone $LZ_m$ . . $LZ_n$ . . $LZ_1$ in such a way that $\text{liquid}_{n+1}$ added to the application zone $LZ_{n+1}$, contacts the flow matrix substantially simultaneously to transport the $\text{liquid}_{n+1}$ through the matrix immediately after $\text{liquid}_n$, added to the nearest downstream application zone $LZ_n$.

The present methods and devices, allowing substantially simultaneous liquid applications but conducting sequential liquid transport in a manner preserving the order of liquid application zones, facilitate automation of analyte determination, avoid the need for sequential addition of sample and analytically detectable reactant, and allow for predeposited analytical reactant for such methodologies. The presently claimed methods and devices are neither taught nor suggested by Dafforn et al.

Dafforn et al disclose a multiple port assay device. Delivery of a sample may be made into the device through a first means or second means using a dropper, syringe needle, etc., resulting in deposit of the sample on a bibulous strip, and a liquid reagent other than

sample may be added to the device. Additional liquid reagents may be added to the device either before or after sample addition, at least one of such reagents being added through the means not used for adding the sample (column 13, lines 32-42). The application of reagents can also be done by breaking an internal liquid-containing container (column 23, line 52).

However, Appellants find no teaching or suggestion by Dafforn et al relating to a method or device as presently claimed wherein at least one biospecific affinity reactant (Reactant I) is firmly anchored in the flow matrix and at least one biospecific affinity reactant is applied to an application zone in combination with a flow matrix arrangement as recited in claims 1 and 18. Particularly, Appellants find no teaching or suggestion by Dafforn et al of a method or device wherein flow is initiated by adding liquid to each zone in such a way that $liquid_{n+1}$ added to the application zone $LZ_{n+1}$ contacts the flow matrix substantially simultaneously with and is transported through the matrix immediately after $liquid_n$, added to the nearest downstream application zone $LZ_n$.

In fact, the only specific mention of simultaneous application which Appellants find in the teachings of Dafforn et al is at column 24, beginning at line 22 wherein an assay is described as conducted by adding a sample suspected of containing human chorionic gonadotrophin (HCG) at a first opening and simultaneously adding a developer solution at the second opening. However, contrary to the present methods and device wherein $liquid_{n+1}$ added to the application zone $LZ_{n+1}$ contacts the flow matrix *substantially simultaneously* with and is transported through the matrix *immediately after* $liquid_n$, added to the nearest downstream application zone $LZ_n$, Dafforn et al disclose that the sample HCG binds to an enzyme conjugate and the resulting complex, namely of HCG and enzyme conjugate, is carried *by the moving developer solution* to the detection zone where it binds, i.e., where the HGC-enzyme conjugate complex binds to the detection zone. Specifically, Dafforn et al disclose:

> The assay can be conducted by adding a sample suspected of containing HCG at the first opening and simultaneously adding a developer solution containing enzyme substrate at the second opening. During subsequent incubation, HCG binds to the conjugate, *the complex is carried by the moving developer to the detection zone* where it binds, and the bound complex acts on the substrate to produce color at the detection zone when HCG is present in the sample (column 24, lines 29-37, emphasis added).

Thus, Appellants find no teaching or suggestion by Dafforn et al that liquid reagent contacts a flow matrix *simultaneously* with a sample and is transported through a matrix *immediately after* the sample. Rather, Dafforn et al teach that HCG-conjugate complex is formed upon addition of sample, and that the thus-formed conjugate is carried *by the moving developer solution* to the detection zone. Dafforn et al provide no teaching or suggestion relating to simultaneous contact and a sequential flow of reagents through a matrix.

Thus, in the present methods and devices, sample and reagent may be applied to the flow matrix simultaneously. The sample begins migration to the detection zone and is followed by liquid migration from the next upstream zone. As a result, there is a continuous migration of sample and reagents through the flow matrix, started by one initial application occasion. The flow of liquids through the flow matrix and the detection zone is in the same order as the liquid application zones. The present methods are advantageous in that sample is provided for reaction in the detection zone, for example with reactant I, prior to contact of sample with the an upstream added liquid, for example, analytically detectable Reactant*. Appellants find no such teachings by Dafforn et al.

The Examiner has asserted that these arguments are not persuasive because the present claims allow n" = n', i.e., the analytically detectable Reactant* liquid application zone $LZ_{n''}R^*$ may be the same as the sample application zone $LZ_{n'}S$. However, Appellants acknowledge that according to claims 1 and 18, the analytically detectable Reactant* liquid application zone $LZ_{n''}R^*$ may be the same as the sample application zone $LZ_{n'}S$, but this does not prevent the claimed method and device from distinguishing over Dafforn et al. That is,

even if the $LZ_{n''}R^*$ and $LZ_{n'}S$ zones coincide, the present claims 1 and 18 still require *at least two liquid application zones in which flow is initiated* and require that flow is initiated by adding liquid to each zone in such a way that liquid added to an upstream zone $LZ_{n+1}$ *contacts the flow matrix substantially simultaneous* with and *is transported through the matrix immediately after* liquid added to the nearest downstream application zone $LZ_n$. Thus, according to claims 1 and 18, if n" = n', there is still yet another liquid application zone which meets the requirements of section II of claim 1 and section e) of claim 18, where sequential transport is obtained, which is not taught by Dafforn et al, even at column 24.

The Examiner has also asserted that there is no support in Dafforn et al for the position that the complex and developer mix with one another and asserted that the addition of developer after the complex would cause the developer to flow behind the complex. The Examiner's first assertion is simply contrary to the teachings of Dafforn et al which state that the complex is *carried by the moving developer to the detection zone* (column 24, lines 33-34). Similarly, the Examiner's second assertion, that the addition of developer after the complex would cause developer to flow behind the complex, is contrary to these specific teachings of Dafforn et al at column 24, that the additions of developer and sample, which forms the complex, are simultaneous. The Examiner's comments appear to be based on the assumption that the device of Dafforn et al could be manipulated to conduct a method as recited in claim 1 or result in a device as recited in claim 18. However, Dafforn et al do not teach any such method or device.

The Examiner appears to have asserted that simultaneous addition of two liquids will inherently result in sequential transport. This, of course, is contrary to the teachings of Dafforn et al at column 24. Moreover, one of ordinary skill in the art will recognize that, as discussed in the present specification, a liquid added in an application zone may have a tendency to spread on top of the matrix to parts of the matrix outside the zone. In fact,

Dafforn et al have such mixing as their objective as the developer carries the complex to the detection zone. In contrast, the present methods and devices require sequential transport through the matrix. Moreover, one skilled in the art will also recognize that when a sample introduction port has additional means for sample treatment, for example filtration of a sample, whole blood separation or the like, the delay in flow to the lateral flow matrix from the sample port results in mixture of the sample with a liquid added upstream of the sample addition port. This may well be the explanation for the disclosed and desired mixing of sample-enzyme conjugate complex and developer of Dafforn et al prior to transport of these components to detection zone.

The Examiner has also asserted that Dafforn et al clearly state at column 24, lines 35-37 that the reaction occurs at the detection zone. In the previously quoted passage, Dafforn et al clearly state that HCG binds to the enzyme conjugate and the complex is carried by the moving developer to the detection zone. Accordingly, the disclosure at column 24, line 35 of "where it binds, and the bound complex acts on the substrate to produce color at the detection zone" refers to binding of the complex with the immobilized second anti-HCG antibody at the detection zone, thereby providing "the bound complex" referenced in the subsequent phrase. There is simply no support for the Examiner's assertion that the complex and developer are not carried together to the detection zone.

Anticipation under 35 U.S.C. §102 requires that each and every element as set forth in the claims is found, either expressly or inherently described, in a single prior art reference, *In re Robertson*, 49 U.S.P.Q.2d 1949, 1950 (Fed Cir. 1999). In view of the deficiencies in the teachings of Dafforn et al with respect to a method or device employing simultaneous contact of at least two liquids with a flow matrix and sequential transport of the liquids through the flow matrix, Dafforn et al do not anticipate the present claims under 35 U.S.C. §102.

Accordingly, the rejection of claims 1, 3, 7, 9, 10, 13, 14, 18, 20, 21, 24, 25, 27, 28 and 32 under 35 U.S.C. §102 should be reversed.

### 3. Claims 3 and 20 Are Independently Patentable

According to claim 3, n"=n' in the method of claim 1, i.e., the application zone for Reactant* coincides with the application zone for sample. Similarly, according to claim 20, n"=n' in the device of claim 18, wherein the application zone for Reactant* coincides with the application zone for sample and the device is intended for simultaneous transport of analyte and Reactant*.

As discussed above, the only teaching Appellants find by Dafforn et al relating to simultaneous application of liquids is the example described at column 24, wherein developer is added to one opening while sample is added to a different opening. Accordingly, the application zones for sample and analytically detectable Reactant* do not coincide. Appellants find no other teaching by Dafforn et al wherein an analytically detectable Reactant* application zone and a sample application zone coincide, particularly with at least one additional liquid application zone and in a method or device employing sequential transport of the additional liquid with respect to sample and analytically detectable Reactant*.

Accordingly, Dafforn et al fail to expressly or inherently describe each and other element as set forth in claims 3 and 20. Thus, Dafforn et al do not anticipate these claims under 35 U.S.C. §102, and the rejection of these claims should be reversed.

### 4. Claim 7 is Independently Patentable.

Claim 7 further defines the method of claim 1 to require that application of liquid is performed simultaneously in all of the liquid application zones.

As discussed above, the only teaching by Dafforn et al which Appellants find relating to simultaneous application of liquids is at column 24 wherein a developer solution and a sample are simultaneously added to respective openings of Dafforn et al's device. However,

in the Dafforn et al example, sequential transport of each liquid is not obtained. Thus, Dafforn et al fail to teach a method according to claim 7 wherein application of liquid is performed simultaneously in all liquid application zones and each liquid contacts the flow matrix substantially with and is transported through the matrix immediately after liquid added to the nearest downstream application zone, as is required by claim 7. In view of the deficiencies in the teachings of Dafforn et al, Dafforn et al do not expressly or inherently describe each and every element as set forth in claim 7 and therefore do not anticipate claim 7 under 35 U.S.C. §102. Accordingly, the rejection of claim 7 should be reversed.

### 5. Claims 10 and 24 are Independently Patentable

Claims 10 and 24 recite the method of claim 1 and the device of claim 18, respectively, wherein the liquid application zones have zone spacers between each other.

Dafforn et al provide no teaching or suggestion of a method or device employing a flow matrix wherein the liquid application zones have zone spacers between each other. The Examiner has asserted that Dafforn et al incorporate dividers as part of the flow matrix between application zones which would act as spacers. However, present claims 1 and 18 recite that the flow matrix comprises liquid application zones and claims 10 and 24 recite that the zones have zone spacers between each other. Thus, the claims require that the dividers are in the flow matrix. In contrast, the "dividers" which the Examiner has asserted are taught by Dafforn et al are the housing portions between sample application wells and Appellants find no dividers provided on a flow matrix in Dafforn et al.

Thus, Dafforn et al do not expressly or inherently describe the limitations of claims 10 and 24 and therefore do not anticipate these claims under 35 U.S.C. §102. The rejection of claims 10 and 24 should therefore be reversed.

### 6. Claim 21 is Independently Patentable

According to claim 21, Reactant* is pre-deposited in its application zone ($LZ_{n''}R^*$) of the device.

Appellants find no teaching or suggestion of such a device wherein an analytically detectable reactant is predeposited in the device and is adapted for sequential flow to a detection zone. To the contrary, Dafforn et al disclose that developer is added to their device and, in the embodiment described at column 24, the developer mixes with liquids added at other points prior to travel to the detection zone.

In view of the deficiencies in the teachings of Dafforn et al, Dafforn et al do not expressly or inherently describe each and every element as set forth in claim 21 and therefore do not anticipate claim 21 under 35 U.S.C. §102. Accordingly, the rejection of claim 21 should be reversed.

## C. The Claims are Nonobvious over Dafforn et al

The methods and devices defined by claims 2, 4, 6, 8, 11, 19, 22 and 23 are nonobvious over and patentably distinguishable from Dafforn et al, whereby the rejection of these claims under 35 U.S.C. §103 should be reversed.

### 1. The Examiner's Rejection

The Examiner rejected claims 2, 4, 6, 8, 11, 19, 22 and 23 under 35 U.S.C. §103 as being anticipated by the Dafforn et al. The Examiner asserted it would have been obvious to Reactant* upstream of a sample application zone and to apply the liquids simultaneously to optimize assay conditions.

### 2. Claims 2 and 19 are Nonobvious Over Dafforn et al

According to claims 2 and 19, n'' > n', wherein the $LZ_{n''}R^*$ and $LZ_{n'}S$ zones do not coincide, whereby flow is initiated by adding liquid to each zone in such a way that liquid added to the upstream Reactant* application zone $LZ_{n''}R^*$ contacts the flow matrix

substantially simultaneous with and is transported through the matrix immediately after liquid added to the nearest downstream application zone, which can be $LZ_{n'}S$.

Dafforn et al provide no teaching or suggestion of a method or device employing such a structure of liquid application zones or of providing sequential transport of simultaneously applied liquids. Column 24 of Dafforn et al, again referenced by the Examiner, teaches simultaneous application of developer solution and sample, but further teaches mixing of the developer solution with sample-containing complex prior to arrival in the detection zone. Thus, Dafforn et al fail to teach or suggest a method or device employing substantially simultaneous application of a liquid in an upstream analytically detectable reactant application zone and sample in a downstream sample application zone, followed by sequential flow of the liquids through the flow matrix.

The Examiner fails to indicate why one of ordinary skill in the art would be motivated to modify either the method or the device taught by Dafforn et al to arrive at the method and device defined by claims 2 and 19. The lack of motivation is particularly critical in view of the fact that the Examiner is suggesting modification in a manner that would prevent the developer solution of Dafforn et al from mixing with the sample-enzyme conjugate complex, a result directly contrary to the teachings of Dafforn et al at column 24. It is error to find obviousness where references diverge from and teach away from the invention at hand, *In re Fine*, 5 U.S.P.Q.2d 1596, 1599 (Fed. Cir. 1988).

In order to render a claimed invention obvious, the prior art must enable one skilled in the art to make and use the claimed invention, *Motorola, Inc. v. Interdigital Tech. Corp.*, 43 U.S.P.Q.2d 1481, 1489 (Fed. Cir. 1997). In view of the failure of Dafforn et al to teach or suggest a method or device employing substantially simultaneous contact of liquids to an upstream analytically detectable reactant application zone and a downstream sample application zone in a flow matrix and sequential flow of the liquids through the flow matrix,

Dafforn et al do not enable one of ordinary skill in the art to make and use the claimed invention. In fact, by teaching mixing of the developer with the HCG-enzyme conjugate complex, Dafforn et al teach away from the presently claimed methods and devices.

Further, the mere fact that prior art could be modified to result in a claimed invention would not have made the modification obvious unless the prior art suggested the desirability of the modification, *In re Mills*, 16 U.S.P.Q.2d 1430 (Fed. Cir. 1990). Appellants find no suggestion by Dafforn et al for modifying their teachings along the lines of the presently claimed methods and devices, nor do Appellants find any suggestion by Dafforn et al regarding the desirability of any such modification. Thus, the present methods and devices are nonobvious over Dafforn et al under 35 U.S.C. §103, and the rejection of claims 2 and 19 should be reversed.

### 3. Claim 4 is Nonobvious Over Dafforn et al

According to claim 4, Reactant* is pre-deposited in its application zone ($LZ_{n}$·R*) in the method of claim 1.

Appellants find no teaching or suggestion of such a method wherein an analytically detectable reactant is predeposited in a liquid application zone and is sequentially transported to a detection zone. To the contrary, Dafforn et al disclose that developer is added to their device and, in the embodiment described at column 24, the developer mixes with liquids added at other points prior to travel to the detection zone.

Appellants find no suggestion by Dafforn et al for modifying their teachings along the lines of the presently claimed method, nor do Appellants find any suggestion by Dafforn et al regarding the desirability of any such modification. Thus, the present method is nonobvious over Dafforn et al under 35 U.S.C. §103, and the rejection of claim 4 should be reversed.

### 4. Claims 6 and 22 are Nonobvious Over Dafforn et al

Claims 6 and 22 specify that $LZ_{n+1}$ is upstream and immediately adjacent $LZ_n$. Thus, these claims require that $liquid_{n+1}$, added to the application zone $LZ_{n+1}$, contacts the flow matrix substantially simultaneously with and is transported through the matrix immediately after $liquid_n$ added to the immediately adjacent downstream application zone $LZ_n$.

As discussed above, the only teaching Appellants find by Dafforn et al relating to simultaneous application of liquids is the example described at column 24, wherein developer is added to one opening while sample is added to a different opening. Appellants find no teaching as to whether or not the liquid application zones are immediately adjacent to one another. However, it is clear from the teachings of Dafforn et al at column 24 that the liquids applied at the respective openings are not sequentially transported to the detection zone, but rather mix in the vicinity of the application areas.

Appellants find no suggestion by Dafforn et al for modifying their teachings along the lines of the presently claimed method and device, nor do Appellants find any suggestion by Dafforn et al regarding the desirability of any such modification. Thus, the present method and device are nonobvious over Dafforn et al under 35 U.S.C. §103, and the rejection of claims 6 and 22 should be reversed.

### 5. Claims 8 and 23 are Nonobvious Over Dafforn et al

Claims 8 and 23 specify that $2 \leq m \leq 6$; n' is 1, 2 or 3; n'' > n; and $LZ_{n'+1}$, $LZ_{n'+2}$, $LZ_{n'+3}$, $LZ_{n'-1}$, and $LZ_{n'-2}$ are application zones for liquids intended for transport of Reactant*, other reactant, or buffer without reactant. Thus, these claims are directed to methods and devices employing a plurality of liquid application zones, wherein respective liquids contact the flow matrix substantially simultaneously and are transported through the matrix immediately sequentially.

As discussed above, the only teaching Appellants find by Dafforn et al relating to simultaneous application of liquids is the example described at column 24, wherein developer is added to one opening while sample is added to a different opening. Appellants find no teaching as to a plurality of liquid application zones as required by claims 8 and 23, particularly wherein substantially simultaneously applied liquids are transported through the matrix sequentially.

Further, Appellants find no suggestion by Dafforn et al for modifying their teachings along the lines of the presently claimed method and device, nor do Appellants find any suggestion by Dafforn et al regarding the desirability of any such modification. Thus, the present method and device are nonobvious over Dafforn et al under 35 U.S.C. §103, and the rejection of claims 8 and 23 should be reversed.

**6. Claim 11 is Nonobvious Over Dafforn et al**

According to claim 11, a composition of liquid flow from an application zone $LZ_n$ is not the same as a composition of liquid flow from the nearest adjacent application zone LZ in which flow is initiated.

While Dafforn et al disclose application of different liquids at the respective first and second openings, Appellants find no teaching or suggestion by Dafforn et al of a method wherein different liquids applied at the respective openings are sequentially transported to the detection zone. Rather, as noted, Dafforn et al disclose that such liquids mix in the vicinity of the application areas.

Appellants find no suggestion by Dafforn et al for modifying their teachings along the lines of the presently claimed method, nor do Appellants find any suggestion by Dafforn et al regarding the desirability of any such modification. Thus, the present method is nonobvious over Dafforn et al under 35 U.S.C. §103, and the rejection of claim 11 should be reversed.

## D. The Claims are Nonobvious over Dafforn et al and Robinson et al

The methods and devices defined by claims 12, 15, 16, 26, 29 and 30 are nonobvious over and patentably distinguishable from Dafforn et al in view of Robinson et al, whereby the rejection of these claims under 35 U.S.C. §103 should be reversed.

### 1. The Examiner's Rejection

Claims 12, 15, 16, 26, 29 and 30 were rejected under 35 U.S.C. §103(a) as being unpatentable over Dafforn et al in view of the Robinson et al published PCT application WO 95/16914. The Examiner relied on Robinson et al as disclosing the use of calibration zones, and the Examiner asserted it would have been obvious to incorporate the calibrator zone as taught by Robinson et al in to the method and device of Dafforn et al.

### 2. Claims 12, 15, 16, 26, 29 and 30 are Nonobvious

Claims 12, 15 and 16 depend directly and indirectly, respectively, from claim 1 while claims 26, 29 and 30 depend directly and indirectly, respectively, from claim 18. Claims 12 and 26 recite that at least one reactant other than Reactant* is pre-deposited in an application zone $LZ_{n'''}R$ for liquid intended for transport of the reactant. According to claim 15, the matrix comprises at least one calibrator zone (CZ), in which calibrator is bound to, or in advance has been bound to, the matrix. According to claim 29, the flow matrix comprises at least one calibrator zone CZ, in which a calibrator or a binder for the calibrator is firmly anchored in the matrix. Claims 16 and 30 recite that the calibrator zone or zones (CZ) of claims 15 and 29, respectively, have a binder for the calibrator firmly anchored in the matrix, the calibrator optionally being pre-deposited in the matrix upstream of the calibrator zone or zones.

The deficiencies of Dafforn et al noted in detail above with respect to claims 1 and 18 apply equally well with respect to claims 12, 15, 16, 26, 29 and 30. Moreover, Appellants find no teaching or suggestion by Dafforn et al relating to an additional zone $LZ_{n'''}R$ as

presently required by claims 12 and 26, or relating to calibration, particularly, integral with their device, or relating to a calibration zone in their device, calibrator predeposited in or applied to a matrix, or a binder for a calibrator in a calibration zone, as required by claims 15, 16, 29 or 30.

These deficiencies of Dafforn et al are not resolved by Robinson et al. Robinson et al describe a sensor device for a sandwich assay comprising a discrete zone having a measurement region on which is immobilized a first specific binding partner for a ligand under assay and a known amount of a releasable optionally labeled second specific binding partner for the ligand under assay, and a second discrete zone having a region on which is immobilized a first specific binding partner for the ligand under assay, a releasable known amount of ligand analog, and a second known amount of a second optionally labeled second specific binding partner for the ligand under assay.

However, Appellants find no teaching or suggestion by Robinson et al of a method or device as recited in claims 1 and 18, respectively, employing at least one analytically detectable biospecific affinity reactant (Reactant*) and at least one firmly anchored biospecific affinity reactant (Reactant I) in a detection zone, with the arrangement of liquid application zones and liquid flows as recited in claims 1 and 18. Additionally, Appellants find no teaching or suggestion for employing any of the elements of Robinson et al's sensor device in the multiple port assay device of Dafforn et al. In fact, while Dafforn et al require application of one or more liquid reagents in addition to a liquid sample through different introduction means, the sensor device of Robinson et al is designed for a sandwich assay wherein only a sample containing a ligand under assay is applied.

Appellants are not claiming the use of additional reactant or calibrator per se. Rather, Appellants are claiming defined methods and devices, in which the flow matrix comprises an additional liquid application zone $LZ_{n'''}R$ (claims 12 and 26) or comprises at least one

calibrator zone (CZ), in which calibrator is bound to, or in advance has been bound to, the matrix (claim 15) or in which a calibrator or a binder for the calibrator is firmly anchored in the matrix (claim 29), and optionally which have a binder for the calibrator firmly anchored in the matrix, the calibrator optionally being pre-deposited in the matrix upstream of the calibrator zone or zones (claims 16 and 30). Appellants find no teaching or suggestion for modifying the teachings of Dafforn et al to include any portion of the Robinson et al teachings, and particularly those which would relate to additional liquid application zones or calibration zones, calibrator and binder as recited in the present claims, to arrive at the invention defined by any of claims 12, 15, 16, 26, 29 or 30.

In order to render a claimed invention obvious, the prior art must enable one skilled in the art to make and use the claimed invention, *Motorola, Inc. v. Interdigital Tech. Corp.*, *supra*. In view of the failure of Robinson et al to resolve the deficiencies of Dafforn et al, particularly with respect to a method and device allowing simultaneous application and sequential transport, or to provide any suggestion for combining and modifying the teachings of Robinson et al and Dafforn et al along the lines asserted by the Examiner, the combination of these references simply does not enable one of ordinary skill in the art to conduct the claimed methods or make and use the claimed devices. Thus, the combination of Dafforn et al and Robinson et al does not render the present claims obvious. It is therefore submitted that the methods and devices defined by claims 12, 15, 16, 26, 29 and 30 are nonobvious over and patentably distinguishable from Dafforn et al and Robinson et al, whereby the rejection under 35 U.S.C. §103 should be reversed.

## E. The Claims are Nonobvious over Dafforn et al and Self

The methods and devices defined by claims 17 and 31 are nonobvious over and patentably distinguishable from Dafforn et al in view of Self, whereby the rejection of these claims under 35 U.S.C. §103 should be reversed.

### 1. The Examiner's Rejection

Claims 17 and 31 were rejected under 35 U.S.C. §103(a) as being unpatentable over Dafforn et al in view of Self. The Examiner relied on Self as disclosing that immunoassays are used for the detection and/or determination of autoimmune diseases. The Examiner concluded it would have been obvious to use the device and method of Dafforn et al for diagnosing autoimmune disease.

Claim 17 depends directly from claim 1 while claim 31 depends directly from claim 18. These claims recite respectively that the method is performed as part of diagnosing allergy or autoimmune disease and that the device is intended for diagnosing allergy or autoimmune disease.

Diagnosing specific allergies or autoimmune diseases, for example IgE antibodies, is often difficult as biological samples such as blood contain a plurality of nonspecific binding members which interfere with reactions necessary for accurate labeling and detection of specific IgE antibodies. Accordingly, the present method and device for diagnosing allergy or autoimmune disease are advantageous in that the sample can reach the detection zone prior to analytically detectable reactant, thereby reducing interference with the analyte-immobilized reactant reaction in the detection zone by the analytically detectable reactants.

The deficiencies of Dafforn et al noted in detail above with respect to claims 1 and 18 apply equally well with respect to claims 17 and 31. Moreover, Appellants find no specific teaching or suggestion by Dafforn et al relating diagnosing allergy or autoimmune disease.

The deficiencies of Dafforn et al are not resolved by Self. That is, while Self discloses an immunoassay using an amplified cyclic detection system, Appellants find no teaching or suggestion by Self relating to a method or device for determination of an analyte in a sample and a flow matrix employing a combination of biospecific affinity reactants and liquid application zones and flow as defined in claims 1 and 18. Similarly, Appellants find no teaching or suggestion by Self for modifying any of the teachings of Dafforn et al to result in either a method or a device as presently claimed. Thus, the mere teaching by Self of the use of immunoassays for detection and/or determination of autoimmune diseases does not resolve the deficiencies of Dafforn et al, particularly with respect to a method and device allowing simultaneous liquid application and sequential liquid transport and the advantages of such with respect to diagnosing allergy or autoimmune disease. Thus, the combination of Dafforn et al and Self does not render the methods and devices of the present claims obvious, and the rejection under 35 U.S.C. §103 should be reversed.

### F. The Claims are Nonobvious over Dafforn et al and Goerlach-Graw et al

The methods and devices defined by claims 34 and 35 are nonobvious over and patentably distinguishable from Dafforn et al in view of Goerlach-Graw et al, whereby the rejection of these claims under 35 U.S.C. §103 should be reversed.

#### 1. The Examiner's Rejection

Claims 34 and 35 were rejected under 35 U.S.C. §103 as being unpatentable over Dafforn et al in view of Goerlach-Graw et al. The Examiner relied on Goerlach-Graw et al as disclosing barriers in the form of strips in a flow matrix. The Examiner asserted it would have been obvious to incorporate such barriers in the device and method of Dafforn et al to avoid flooding of test elements with sample liquid.

### 2. Claims 34 and 35 are Nonobvious

Claim 34 depends directly from claim 10, and therefore claim 1, while claim 35 depends directly from claim 24, and therefore claim 18. These claims recite that each zone spacer (between the liquid application zones) comprises a strip attached to the flow matrix.

The deficiencies of Dafforn et al noted in detail above with respect to claims 1 and 18 and claims 10 and 24 apply equally well with respect to claims 34 and 35. Moreover, Appellants find no specific teaching or suggestion by Dafforn et al relating zone spacers comprising a strip attached to the flow matrix. In fact, placement of spacers between liquid application zones on the flow matrix of Dafforn et al would be contrary to the teachings of Dafforn et al which desire contact of developer and the analyte-enzyme conjugate complex prior to the detection zone.

The deficiencies of Dafforn et al are not resolved by Goerlach-Graw et al. That is, Goerlach-Graw et al disclose a device including three individual test strips extending in parallel from a single application zone. However, Appellants find no teaching in this reference relating to a single flow matrix having liquid application zones in series therein, as required in claims 1 and 18, particularly with spacers in the form of a strip between each zone. While the Examiner refers to column 6, the various embodiments at column 6 are directed to slowing liquid migration in one or more of the strips so that there is simultaneous wetting of the sample withdrawal sites on the respective test strips. This provides no teaching or suggestion of zone spacers as presently claimed between a plurality of liquid application zones in series in a single flow matrix, or for modifying the teachings of Dafforn et al along the lines of the present invention. In fact, such test strips are contrary to promoting contact between developer and conjugate as desired by Dafforn et al.

Thus, the combination of Dafforn et al and Goerlach-Graw et al does not resolve the deficiencies of Dafforn et al, particularly with respect to a method and device allowing

simultaneous liquid application and sequential liquid transport, and therefore does not render the methods and devices of the present claims obvious. Accordingly, the rejection under 35 U.S.C. §103 should be reversed.

## IV. CONCLUSIONS

Thus, the methods defined by claims 1-4 and 6-17 are definite to one of ordinary skill in the art, and the methods, devices and test kits defined by claims 1-4 and 6-35 are neither anticipated by nor rendered obvious over Dafforn et al or the combination of Dafforn et al with any of Robinson et al, Self or Goerlach-Graw et al. Accordingly, the rejections under 35 U.S.C. §§ 102, 103 and 112, second paragraph, should be reversed. Favorable action by the Board is respectfully requested.

Respectfully submitted,

Holly D. Kozlowski

Holly D. Kozlowski
Registration No. 30,468
Dinsmore & Shohl LLP
1900 Chemed Center
255 East Fifth Street
Cincinnati, Ohio 45202
(513) 977-8568

## APPENDIX

1. A method for determination of an analyte in a sample in a flow matrix by use of a transport flow of one or more biospecific affinity reactants, at least one of which is analytically detectable (Reactant*) and one of which is firmly anchored in the matrix (Reactant I), and the flow matrix comprises:

A) an application zone adapted for application of liquid (LZ), which liquid contains buffer and sample and optionally reactants needed for a complete determination, but not Reactant I,

B) a detection zone (DZ) with the firmly anchored reactant (Reactant I) located downstream of LZ, and

C) optionally one or more zones in which any of the reactants needed for a complete determination, but not Reactant I, has been pre-deposited,

wherein (i) the flow towards the detection zone is initiated by addition of the liquid with sample in the application zone LZ for transport of analyte and reactants towards the detection zone (DZ), and (ii) the amount of the Reactant* bound to DZ is detected, wherein the detected amount is correlated to the amount of analyte in the sample, wherein

I. the flow matrix comprises at least two application zones for liquid LZ arranged substantially adjacent to each other:

$$LZ_m \ldots LZ_n \ldots LZ_1 \qquad DZ$$

$$\longrightarrow$$

flow direction $\longrightarrow$

wherein

a) $LZ_n$ is an application zone for liquid, and n is the position of the application zone $LZ_n$,

b) m is the total number of application zones in which flow is initiated, m is greater than or equal to 2, and m is not equal to n, wherein $LZ_m$ is the farthest upstream liquid application zone,

c) one $LZ_n$ is an application zone for sample ($LZ_{n'}S$) and one $LZ_n$ is for Reactant* ($LZ_{n''}R^*$) with $n'' \geq n'$;

d) $\longrightarrow$ is the direction of the flow, and

e) DZ is the detection zone, and

II. flow is initiated by adding liquid to each zone $LZ_m$ . . $LZ_n$ . . $LZ_1$ in such a way that $liquid_{n+1}$, added to the application zone $LZ_{n+1}$, contacts the flow matrix substantially simultaneously with and is transported through the matrix immediately after $liquid_n$ added to the nearest downstream application zone $LZ_n$.

2. The method according to claim 1, wherein $n'' > n'$.

3. The method according to claim 1, wherein $n'' = n'$.

4. The method according to claim 1, wherein Reactant* is pre-deposited in its application zone ($LZ_{n''}R^*$).

6. The method according to claim 1, wherein $LZ_{n+1}$ is upstream and immediately adjacent $LZ_n$.

7. The method according to claim 1, wherein application of liquid is performed simultaneously in all $LZ_m$ . . $LZ_n$ . . $LZ_1$.

8. The method according to claim 1, wherein $2 \leq m \leq 6$; n' is 1, 2 or 3, n" > n'; $LZ_{n'+1}$, $LZ_{n'+2}$, $LZ_{n'+3}$, $LZ_{n'-1}$, and $LZ_{n'-2}$ are application zones for liquids intended for transport of Reactant* or other reactant or buffer without reactant.

9. The method according to claim 1, wherein at least one of the zones $LZ_m$ . . $LZ_n$ . . $LZ_1$ comprises a pad or material layer applied on the flow matrix.

10. The method according to claim 1, wherein the zones $LZ_m$ . . $LZ_n$ . . $LZ_1$ have zone spacers between each other.

11. The method according to claim 1, wherein a composition of liquid flow from an application zone $LZ_n$ is not the same as a composition of liquid flow from the nearest adjacent application zone LZ in which flow is initiated.

12. The method according to claim 1, wherein at least one reactant, other than Reactant*, is pre-deposited in an application zone $LZ_n$...R for liquid intended for transport of the reactant.

13. The method according to claim 1, wherein $2 \leq m \leq 6$ and n' for the application zone for sample ($LZ_{n'}S$) is 1, 2 or 3.

14. The method according to claim 1, wherein Reactant* has biospecific affinity for the analyte so that Reactant* is incorporated into a complex Reactant'---Analyte---Reactant*

in the detection zone in an amount related to the amount of analyte in the sample, in which complex Reactant' has biospecific affinity to the analyte and is

(a) Reactant I, or

(b) a reactant to which Reactant I exhibits biospecific affinity and which is transported from $LZ_{n'}S$ or from an application zone downstream of $LZ_{n'}S$.

15. The method according to claim 1, wherein the matrix comprises at least one calibrator zone (CZ), in which calibrator is bound to, or in advance has been bound to the matrix.

16. The method according to claim 15, wherein the calibrator zone or zones (CZ) have a binder for the calibrator firmly anchored in the matrix, the calibrator optionally being pre-deposited in the matrix upstream of the calibrator zone or zones.

17. The method according to claim 1, wherein the method is performed as part of diagnosing allergy or autoimmune disease.

18. A device for determination of an analyte in a sample in a flow matrix by use of a transport flow of one or more biospecific affinity reactants, at least one of which is analytically detectable (Reactant*) and one of which is firmly anchored in the matrix (Reactant I), said device comprising a flow matrix having:

A) an application zone for liquid (LZ) containing buffer and sample and optionally reactants needed for a complete determination, but not Reactant I,

B) a detection zone (DZ) with the firmly anchored reactant (Reactant I) located downstream of LZ, and

C) optionally one or more zones in which any of the reactants has been pre-deposited,

wherein

the flow matrix comprises at least two application zones for liquid arranged substantially adjacent to each other:

$$LZ_m \ldots LZ_n \ldots LZ_l \qquad DZ$$

$$\xrightarrow{\hspace{12em}}$$

flow direction $\longrightarrow$

wherein

a) $LZ_n$ is an application zone for liquid, and n is the position of the application zone $LZ_n$,

b) m is the total number of application zones in which flow is initiated, m is greater than or equal to 2, and m is not equal to n, wherein $LZ_m$ is the farthest upstream liquid application zone,

c) one $LZ_n$ is an application zone for sample ($LZ_{n'}S$) and one $LZ_n$ is for Reactant* ($LZ_{n''}R^*$) with $n'' \geq n'$;

d) $\longrightarrow$ is the direction of the flow, and

e) DZ is the detection zone, wherein the device is adapted, when flow is initiated by adding liquid to each zone $LZ_m$ . . $LZ_n$ . . $LZ_l$ in such a way that $liquid_{n+1}$ added to the application zone $LZ_{n+1}$, contacts the flow matrix substantially simultaneously with and is transported through the matrix immediately after $liquid_n$, added to the nearest downstream application zone $LZ_n$.

19. The device according to claim 18, wherein n" > n' and the device is intended for sequential transport of analyte and Reactant*.

20. The device according to claim 18, wherein n" = n' and the device is intended for simultaneous transport of analyte and Reactant*.

21. The device according to claim 18, wherein Reactant* is pre-deposited in its application zone ($LZ_{n''}R^*$).

22. The device according to claim 18, wherein $LZ_{n+1}$ is upstream and immediately adjacent $LZ_n$.

23. The device according to claim 18, wherein $2 \leq m \leq 6$; n' is 1, 2 or 3; n'' > n; $LZ_{n'+1}$, $LZ_{n'+2}$, $LZ_{n'+3}$, $LZ_{n'-1}$, and $LZ_{n'-2}$ are application zones for liquids intended for transport of Reactant* or other reactant or buffer without reactant.

24. The device according to claim 18, wherein the zones $LZ_m$ . $LZ_n$ . $LZ_1$ have zone spacers between each other.

25. The device according to claim 18, wherein at least one of the zones $LZ_m$ . . $LZ_n$ . . $LZ_1$ comprises a pad or material layer applied on the flow matrix.

26. The device according to claim 18, wherein at least one reactant, other than Reactant*, is pre-deposited in an application zone $LZ_{n'''}R$ for liquid intended for transport of the reactant.

27. The device according to claim 18, wherein $2 \leq m \leq 6$ and n' for the application zone for sample ($LZ_{n'}S$) is 1, 2 or 3.

28. The device according to claim 18, wherein the detection zone DZ comprises firmly anchored Reactant I, and a reactant to which Reactant I exhibits biospecific affinity optionally is pre-deposited in $LZ_{n'}S$ or in an application zone downstream of $LZ_{n'}S$.

29. The device according to claim 18, wherein the flow matrix comprises at least one calibrator zone CZ, in which a calibrator or a binder for the calibrator is firmly anchored in the matrix.

30. The device according to claim 29, wherein the calibrator zone or zones (CZ) have a binder for the calibrator firmly anchored in the matrix, and calibrator optionally is pre-deposited in the matrix upstream of the calibrator zone or zones.

31. The device according to claim 18, wherein the device is intended for diagnosing allergy or autoimmune disease.

32. A test kit, comprising (i) a device according to claim 18, and (ii) Reactant*.

33. The test kit according to claim 32, wherein the kit additionally comprises (iii) a calibrator when a binder for the calibrator is firmly anchored in the matrix.

34. The method according to claim 10, wherein each zone spacer comprises a strip attached to the flow matrix.

35. The device according to claim 24, wherein each zone spacer comprises a strip attached to the flow matrix.

1058413